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Group Art Unit No.: 1648

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## CROSS REFERENCE TO RELATED APPLICATIONS

This application is a 371 of PCT/EP98/08563, filed December 18, 1998, which claims priority from GB 9727262.9, filed December 24, 1997.

## BACKGROUND OF THE INVENTION

Papillomaviruses are small naked DNA tumour viruses (7.9 kilobases, double strand), which are highly species-specific. Over 70 individual human papillomavirus (HPV) genotypes have been described. Papillomaviruses are classified on the basis of species of origin (human, bovine etc.) and of the degree of genetic relatedness with other papillomaviruses from the same species. HPVs are generally specific for the skin or mucosal surfaces and have been broadly classified into "low" and "high" risk viruses. Low risk HPVs usually cause benign *lesions* (warts or papillomas) that persist for several months or years. High risk HPVs are associated with pre-neoplastic lesions and cancer. The strongest positive association between an HPV virus and human cancer is that which exist between HPV 16 and 18 and cervical carcinoma. More than ten other HPV types have also been found in cervical carcinomas including HPV 31 and HPV 33 although at less frequency.

Genital HPV infection in young sexually active women is common and most individuals either clear the infection, or if lesions develop, these regress. Only a subset of infected individuals has lesions which progress to high grade intraepithelial neoplasia and only a fraction of these progress further to invasive carcinoma.

The molecular events leading to HPV infection have not been clearly established. The lack of an adequate *in vitro* system to propagate human papillomaviruses has hampered the progress to a best information about the viral cycle.

Today, the different types of HPVs have been isolated and characterised with the help of cloning systems in bacteria and more recently by PCR amplification. The molecular organisation of the HPV genomes has been defined on a comparative basis with that of the well characterised bovine papillomavirus type 1 (BPV1).

Although minor variations do occur, all HPVs genomes described have at least seven early genes, E1 to E7 and two late genes L1 and L2. In addition, an upstream regulatory region harbors the regulatory sequences which appears to control most transcriptional events of the HPV genome.

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E1 and E2 genes are involved in viral replication and transcriptional control, respectively and tend to be disrupted by viral integration. E6 and E7 are involved in viral transformation. E5 has also been implicated in this process.

In the HPVs involved in cervical carcinoma such as HPV 16 and 18, the oncogenic process starts after integration of viral DNA. The integration results in the inactivation of genes coding for the capsid proteins L1 and L2 and loss of E2 repressor function leads to deregulation of the E6/E7 open reading frame installing continuously overexpression of the two early proteins E6 and E7 that will lead to gradually loss of the normal cellular differentiation and the development of the carcinoma. E6 and E7 overcome normal cell cycle by inactivating major tumor suppressor proteins, p53 and pRB, the retinoblastoma gene product, respectively.

Carcinoma of the cervix is common in women and develops through a pre-cancerous intermediate stage to the invasive carcinoma which frequently leads to death. The intermediate stages of the disease is known as cervical intraepithelial neoplasia and is graded I to III in terms of increasing severity (*CIN I-III*).

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Clinically, HPV infection of the female anogenital tract manifests as cervical flat condylomas, the hallmark of which is the koilocytosis affecting predominantly the superficial and intermediate cells of the cervical squamous epithelium.

Koilocytes which are the consequence of a cytopathic effect of the virus, appear as multinucleated cells with a perinuclear clear haloe. The epithelium is thickened with abnormal keratinisation responsible for the warty appearance of the lesion.

Such flat condylomas when positive for the HPV 16 or 18 serotypes, are high-risk factors for the evolution toward cervical intraepithelial neoplasia (CIN) and carcinoma in situ (CIS) which are themselves regarded as precursor lesions of invasive cervix carcinoma.

The natural history of oncogenic HPV infection presents three consecutive phases, namely:

- (1) a latent infection phase,
- (2) a phase of intranuclear viral replication with product of complete virions, which corresponds to the occurrence of koilocytes. At this stage, the HPV is producing its full range of proteins including E2, E5, E6, E7, L1 and L2.
- (3) a phase of viral integration into the cellular genome, which triggers the onset of malignant transformation, and corresponds to CIN II and CIN III/CIS with progressive

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disappearance of koilocytes. At this stage, the expression of E2 is down-regulated, the expression of E6 and E7 is enhanced. Between CIN II/III and CIN III / Cervix carcinoma the viral DNA changes from being episomal in the basal cells to integration of E6 and E7 genes only (tumoral cells). 85% of all cervix carcinomas are squamous cell carcinomas most predominantly related to the HPV16 serotype. 10% and 5% are adenocarcinomas and adenosquamous cell carcinomas respectively, and both types are predominantly related to HPV 18 serotype. Nevertheless other oncogenic HPV's exist.

International Patent Application No. WO 96/19496 discloses variants of human papilloma virus E6 and E7 proteins, particularly fusion proteins of E6/E7 with a deletion in both the E6 and E7 proteins. These deletion fusion proteins are said to be immunogenic.

Immunomodulatory oligonucleotides contain unmethylated CpG dinucleotides ("CpG") and are known (WO 96/02555, EP 468520). CpG is an abbreviation for cytosine-guanosine dinucleotide motifs present in DNA. Historically, it was observed that the DNA fraction of BCG could exert an anti-tumour effect. In further studies, synthetic oligonucleotides derived from BCG gene sequences were shown to be capable of inducing immunostimulatory effects (both in vitro and in vivo). The authors of these studies concluded that certain palindromic sequences, including a central CG motif, carried this activity. The central role of the CG motif in immunostimulation was later elucidated in a publication by Krieg, Nature 374, p546 1995. Detailed analysis has shown that the CG motif has to be in a certain sequence context, and that such sequences are common in bacterial DNA but are rare in vertebrate DNA.

It is currently believed that this evolutionary difference allows the vertebrate immune system to detect the presence of bacterial DNA (as occurring during an infection) leading consequently to the stimulation of the immune system. The immunostimulatory sequence as defined by Krieg is:

Purine Purine CG pyrimidine pyrimidine and where the CG motif is not methylated. In certain combinations of the six nucleotides a palindromic sequence is present. Several of these motifs, either as repeats of one motif or a combination of different motifs, can be present in the same oligonucleotide. The presence of one or more of these immunostimulatory sequence containing oligonucleotides can activate various immune subsets, including natural killer cells (which produce interferon  $\gamma$  and have cytolytic activity) and macrophages (Wooldrige et al Vol 89 (no. 8), 1977). Although other unmethylated CpG

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containing sequences not having this consensus sequence have now been shown to be immunomodulatory.

#### BRIEF SUMMARY OF THE INVENTION

The present invention relates to vaccine compositions, comprising an E6 or/ and E7 or E6, E7 fusion protein from an HPV strain optionally linked with an immunological fusion partner and formulated with a CpG containing oligonucleotide into vaccines that find utility in the treatment or prophylaxis of human papilloma virus induced tumours or lesions.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 and 2 illustrate the therapeutic potential of a vaccine containing the PD1/3 E7 fusion protein and different CpG oligonucleotides as assessed by the tumour growth in the TC1 model (E7 expressing tumour model). The mean tumour growth (in mm<sup>2</sup>/group n=10 mice) over a period of 4 weeks is represented.

Figures 3 and 4 show the relative percentage of the different IgG isotypes (IgG1, IgG2a, IgG2b, IgGTot) in the total of IgGs as measured by ELISA, 2 and 4 weeks post II respectively. Group 1 received PBS, group 2 received ProtD1/3 E7 HPV16, group 5 received ProtD1/3 E7 HPV16 + oligo 1, group 6 received Oligo 1, group 7 received ProtD1/3 E7 HPV16 + oligo 2 and group 8 received Oligo 2.

Figure 5 illustrates the tumour regression as measured by the mean tumour growth (per group of 5 animals) in animals immunised with a vaccine containing the PD1/3 E7 fusion protein and different phosphorothioate modified CpG oligonucleotides. 10<sup>6</sup> TC1 cells were injected subcutaneously (200µl) in the flank of immunocompetent C57BL/6 mice, mice have been vaccinated twice intra- footpad (100 µl : 50µl / footpad), 7 and 14 days after the tumour challenge, with 5µg ProtD 1/3 E7 HPV16.

Figure 6 shows that therapeutic vaccination with CpG oligonucleotide and PD1/3 E7 fusion protein results in a reduction of tumour growth and can induce complete tumour regression, as assessed in the transgenic mice expressing E7 protein.